



Genetic diversity and introductions patterns of *Lantana camara* L. in the territory of Bangladesh as revealed by microsatellite profiles

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ABSTRACT

Lantana camara L. is one of the worst invasive species associated with biodiversity loss and ecosystem desolation. The present study aimed to understand the pattern of introduction and the genetic diversity of *L. camara* populations in Bangladesh to gain insight into the hypothesis concerning the role of multiple introductions in genetic diversity elevation. Plants were collected from different locations under the major administrative divisions namely Dhaka, Sylhet, Rajshahi, Mymensingh and Chattogram of Bangladesh. Genomic DNA was extracted using CTAB extraction buffer. Presence of private alleles and high genetic diversity was revealed by analysis of 8 microsatellites with a total of 30 alleles from 17 samples. Higher (79%) among population genetic variation, multiple divergent genetic clusters and a significant positive correlation between genetic and geographic distances along with moderately strong signal of isolation by distance delineated that multiple introduction of *L. camara* L. population occurred in different

geographical regions of Bangladesh followed by a distance limited gene flow which resulted into the increased genetic diversity of lantana populations. The result of the present study is pertinent with and supports the hypothesis that multiple introduction contributes in gaining genetic diversity of invasive species in introduced range.

Keywords: Invasion, introduction, molecular marker, polymorphism, population genetics.

1. INTRODUCTION

Invasion of natural communities by alien plant species has become a serious threat to biodiversity and ecosystems worldwide (Seabloom et al. 2006; SaxandGaines 2008) since it causes erosion of genetic diversity and disruption of ecosystem processes (Pysek and Richardson 2010). Thus, invasive species exerts serious conservational and ecological impacts on their introduced ranges (Hejda et al. 2009; Vila et al. 2011). Nevertheless, proper management and control of invasive exotic species requires enhanced knowledge about the introduction patterns and genetic structure of the invasive plant species.

Invasive alien species undergo rapid genetic changes (Sakai et al. 2001; Maron et al. 2004) and adaptation to local conditions as an important form of evolution (Reznick and Ghalambor 2001) soon after invading a new geographical region. Genetic variability plays an important role in determining a population's potential to become invasive (Kolbe et al. 2004; Roman and Darling 2007; Lavergne and Molofsky 2007; Facon et al. 2008). Multiple introductions (Novak and Mack 2005; Rosenthal et al. 2008; Paironet et al. 2010; Kelageret et al. 2013) can reduce genetic bottleneck and contribute in regaining high genetic variability and thereby can improve the evolutionary potential of introduced populations (Marrs et al. 2008; Hufbauer 2008; Henry et al. 2009) resulting in successful invasion. Following multiple introduction, admixture or intraspecific hybridization and gene flow may occur contributing in successful invasion (Marrs et al. 2008). Some studies hypothesized on contribution of multiple introductions in gaining genetic diversity as well as invasion success of invasive in introduced areas (Kolbe et al. 2004; Novak and Mack 2005), whereas, other studies hypothesized that multiple introduction is not necessarily the main cause for elevating genetic diversity, as a high genetic diversity in an invasive species population was found as a consequence of a single introduction event containing several genotypes (Eales et al. 2010) and possibly phenotypic plasticity served for the spread of the invasive species. As it is still disputable whether multiple introductions of invasive species from genetically different lineages followed by admixture and gene flow can lead to increased heterozygosity or novel combinations of alleles in the new range and invasion success or not, therefore, more evidence is required to prove the hypothesis regarding introduction pattern and genetic diversity of invasive species.

Lantana (*Lantana camara* L.), native to Central and South America, has been reported as one of the most widespread invasive alien species around the world (Richardson and Rejmanek 2011). During the later part of the nineteenth century, this species was introduced in Indian sub-continent and then a number of cultivars were disseminated throughout the sub-continent (Howard 1970). In Bangladesh, several reports showed that *L. camara* L. is aggressively growing much faster than native species in forests, agricultural land, tea garden and wastelands reducing native associated species (Aktar and Zuberi 2009). However, in spite of its invasiveness, there have been no data on the population genetics particularly introduction patterns, establishment and genetic diversity of *L. camara* L. in the territory of Bangladesh. Therefore, we utilized the opportunity to study lantana invasion in Bangladesh to gain further insight into genetic divergence of an invasive species in an introduced range, its pattern of introduction and relation to invasion success.

Determination of genetic variability is required not only to diagnose a population's capacity to become invasive but also to reveal the invasion histories, processes and dispersal of invaders in new area and future scenario of invasion of the invaded region (Sakai et al. 2001; Prentis et al. 2008; Hagenblad et al. 2015). Use of molecular markers is useful tool to reveal the insights on the genetic diversity and population structure of the organisms. Microsatellites, or simple sequence repeats (SSRs), have been used in population genetic studies in tropical forest tree species (Pandey and Geburek 2009). The present study, therefore, aimed to investigate current status of genetic variability of *L. camara* L. populations by using microsatellite profiles and to demonstrate whether single or multiple introductions happened in the territory of Bangladesh. The findings of the study are also expected to substantiate the hypothesis regarding contribution of introduction pattern in gaining enriched genetic diversity and successful invasion.

2. MATERIALS AND METHODS

2.1. Description of study sites and collection of sample

In this study, five administrative divisions Dhaka, Mymensingh, Sylhet, Chattogram and Rajshahi were selected to collect plant samples for molecular analysis. These sampling sites were chosen based on several reports of invasion by *L. camara* L. (Aktar and

Zuberi 2009; Parvez and Akhter 2010; Rahman and Roy 2014; Dutta et al. 2015). Five populations of *Lantana* were collected from five divisions and named after the divisions and each population consisted of 5-3 samples. A total of 17 plant samples, five from Dhaka and each three from Mymensingh, Sylhet, Chattogram and Rajshahi were collected from locations those were at least 50 m away from each other within a division. The samples were collected from Savar, Muktagacha, Gowainghat, Shitakundo and Rajshahi city under the divisions Dhaka, Mymensingh, Sylhet, Chattogram and Rajshahi, respectively (Fig. 1, Table 1). The locations of samples showed a wide range of geographical distribution within the territory of Bangladesh ranging from 22° 36' 10.26" N to 24° 59' 50.1" N latitude and from 88° 38' 16.5912" E to 91° 56' 11.4" E longitude (Table 1). However, no samples were collected from southern part of the country as there is no invasion record of *lantana* in salinity prone southern part of Bangladesh as because *L. camara* L. is reported to be susceptible to saline soils (Dayet al. 2003).

Fully expanded youngest fresh leaves were collected from *lantana* plants. Leaf sample was collected during the months of March and April in 2017. After collection from the field, leaves were brought to the laboratory and stored at -20 °C until DNA extraction was done.

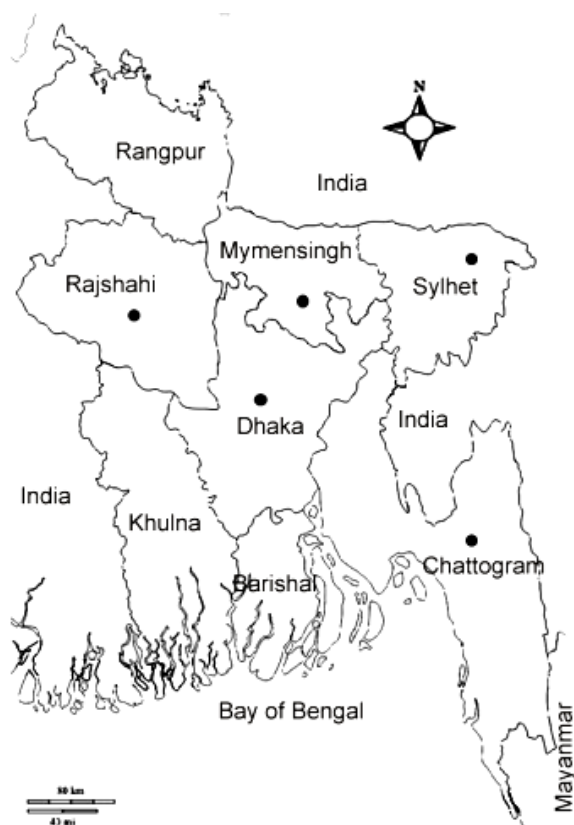


Figure 1. Geographic locations from which *Lantana camara* L. specimens were collected for this study (round marked). The numbers of localities are enlisted in Table 1.

Table 1 Geographic information of the locations of sampling sites

Populations	Locality	Latitude	Longitude
Dhaka	Savar	23°52'46.02" N	90° 16' 8.04" E
	Savar	23°52' 44.076" N	90° 16' 4.8" E
	Savar	23° 52' 25.5" N	90° 15' 55.08" E
	Savar	23° 53' 9.888" N	90° 16' 4.08" E
	Curzon hall	23° 43' 37.38" N	90° 24' 5.76" E
Sylhet	Gowainghat	24° 59' 50.1" N	91° 56' 11.4" E
	Gowainghat	24°59' 41.748" N	91° 55' 55.92" E
	Gowainghat	24° 58' 45.84" N	91° 54' 29.88" E
Chattogram	Sitakundo	22° 36' 10.26" N	91° 40' 32.88" E

	Sitakundo	22°36'40.2372"N	91° 39' 58.86" E
	Sitakundo	22° 36' 38.88" N	91° 39' 51.12" E
Mymensingh	Muktagacha	24° 43' 49.44" N	90° 23' 35.7" E
	Muktagacha	24°43' 48.936" N	90° 23' 35.808" E
	Muktagacha	24°43' 49.656" N	90° 23' 34.332" E
Rajshahi	Rajshahi city	24°22'20.7768"N	88°38' 18.3336" E
	Rajshahi city	24°22'19.0632"N	88°38' 16.5912" E
	Rajshahi city	24°22'19.9596"N	88° 38'18.3948" E

2.2. DNA isolation, PCR amplification and Gel electrophoresis

A modified 2% CTAB extraction protocol (Allen et al. 2006) was used to isolate total genomic DNA. For this, 200-250mg fresh leaf was grounded in liquid nitrogen. The integrity of the isolated DNA was verified by visualizing the extracted DNA on 1% agarose gel stained with ethidium bromide buffered with $1 \times$ TAE. The extracted DNA samples were then stored at -20°C until subsequent PCR amplification.

For PCR amplification, eight nuclear microsatellite loci standardized for *L.camara* L. was used (Ray et al. 2012). Amplification was carried out in a reaction volume of 25 μl in total for each sample containing 50ng of template DNA, 12.5 μl of PCR master mix containing 1 mM of each dNTP, 1.5mM MgCl_2 , 1unit *Taq* polymerase, 1 μl 100 pico moles of primer (0.5 μl of each forward and reverse sequence) and 8.5 μl of sterile de-ionized distilled PCR water. PCR amplification was done in an oil-free thermal cycler (Applied Biosystems 2720 Thermal Cycler). Initial PCR condition was 94°C for 3 minute followed by 35 cycles of denaturation at 94°C for 1 minute, annealing temperature for 30 seconds then extension at 72°C for 1 minute and final elongation step of 72°C for 10 minutes was added.

The amplified products were separated electrophoretically on 2.5% agarose gel prepared using 100 ml $50\times$ TAE buffer and containing 0.5 $\mu\text{g}/\text{ml}$ ethidiumbromide. Agarose gel electrophoresis was conducted in $50\times$ TAE buffer at 100 Vand 300 mA for 1 hour. One molecular weight marker 1.0 kb Plus DNA ladder was electrophoresed alongside the reaction samples. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system (Cleaver Scientific's MultiSUBTM).

2.3. Microsatellite data analysis

Owing to the polyploid nature of *L. camara* L., the number of alleles detected per individual varied by locus and in case of polyploidy genotype as it is difficult to determine the number of copies of an allele in heterozygotes, therefore, banding patterns observed at a particular locus were recorded as a presence(1) or absence(0) matrix which are referred to as 'allele phenotypes' (Saltonstall 2003). Several measures of genetic diversity including allelic richness as the total number of allele per locus and per population were calculated. The mean effective number of alleles (N_e), expected heterozygosity (H_e) for all loci per population, number of private allele and the percentage (%) of polymorphic loci for each population were calculated. Associations among the individuals from different populations were assessed by Principle Coordinate Analysis (PCoA) and a hierarchical Analysis of Molecular Variance (AMOVA) (Armstrong and De Lange 2005) using a pair wise, individual-by individual genetic distance matrix among molecular loci was performed to partition the overall molecular variation into within and among population variances. All these analysis were performed by using the software GenAlex6.5 (Peakall and Smouse2012).

Nei's gene diversity (Nei 1973), Shannon's Information index (Lewontin, 1972), Nei's Measures of Genetic Identity and Genetic distance (Nei 1978) was measured using Popgene version 1.32 software (Yeh et al. 1999). A measure of Isolation By Distance (IBD) was obtained by plotting genetic distance values against log transformed geographic distance values and by performing a Mantel test using the software GenAEx 6.5. DMS (Degrees-minutes-seconds) coordinate values for each of the sampling sites were converted to UTM (Universal Transverse Mercator) coordinate, these units are in meters. Then, to calculate the geographic distance among the sampling sites, these UTM values were converted into Km and used to create a log transformed distance matrix before using in the analysis.

3. RESULTS

3.1 Genetic diversity analysis

Among eight microsatellite markers used in the present study, seven were found highly polymorphic excepting one (CT8) which showed no band. The polymorphic seven markers are produced 2-10 alleles per locus (Table 2) with a total of 30 alleles of which all showed polymorphism demonstrating a 100% polymorphism. The highest number of polymorphic band (10) was found in Dhaka

population showing 33.33% polymorphism (Table 3). Allelic richness was quite high inspite of small number of samples per population with the highest in Rajshahi (14) followed by Dhaka, Chattogram and Mymensingh populations each with same richness (11) and Sylhet (8) over all loci.

The mean effective number of allele ranged from 1.241 to 1.032 in different populations for all locus. The mean expected heterozygosity ranged from 0.131 ± 0.037 to 0.016. Other diversity indices like Shanon's information index varied from 0.1841 to 0.0424 per population and overall population Shanon's information index was 0.517. Nei's gene diversity was found ranging from 0.123 to 0.0148 per population and it was 0.341 for overall population (Table 3).

A total of 16 private alleles were found in total 17 samples and the number varied among the populations (Table 3). The highest number of private allele (5) was found in Rajshahi population followed by Dhaka, Sylhet and Mymensingh with the same number (3) and Chattogram (2).

Table 2 Locus, repeat motif, primer sequences, A_R = allelic richness (total number of allele) per locus for seven standardized microsatellites in *Lantana camara* L.

Locus	Repeat motif	Primer sequence		Allelic richness (A_R)	Genebank Accession
		Left sequence	Right sequence		
Lac4	(AAC)5	CCTACAGCCGAACGACCTAC	TGCTTCGCCCTTTCTGTTAC	4	JN043466
Lac5	(CTT)8	GCTTCGACACGTATCCACT	GGTTGAGGATTCGGTGAATG	10	JN043467
Lac6	(GA)7	AGGACCAATCATTCCCATCA	AGATCTTGGGCAGGACCTTT	3	JN043468
Lac11	(AG)8	CGGCTAAGTTCAGCTTTGT	CGTTGTGTGGCCCTATTTTC	5	JN043472
Lac14	(GT)7	CGCCCTGTTTCTTTAGGT	CCTACCAACTGCCAAAGCAC	2	JN043471
Phc17	(TC)14	GGAGGCCGTTTCTTTGTTTT	CCAAAGAATATGCTGATCAAAGAG	4	EU286562
Phc18	(ATG)6	TGGTCCTGATGGCATTITT	CCCAGAGCGAGCTTAATCAG	2	EU286561

Table 3 Diversity statistics (A_R = allelic richness; N_e = effective number of allele; H_e = expected heterozygosity; I^* = Shanon's information index; h^* = Nei's gene diversity; %P= percentage of polymorphic loci; number of polymorphic loci and number of private alleles) of five *Lantana camara* L. populations and combined population over all loci.

Population	Sample size	A_R	N_e (Mean \pm SE)	H_e (Mean \pm SE)	I^* (Mean \pm SE)	h^* (Mean \pm SE)	%P	No. of polymorphic band	No. Private allele
Total	17	30	1.105 ± 0.023	0.056 ± 0.012	0.517 ± 0.138	0.341 ± 0.123	100%	30	16
Dhaka	5	11	1.241 ± 0.072	0.131 ± 0.037	0.1841 ± 0.268	0.123 ± 0.181	33.33%	10	3
Sylhet	3	8	1.078 ± 0.045	0.043 ± 0.024	0.064 ± 0.194	0.044 ± 0.135	10.00%	3	3
Chattogram	3	11	1.064 ± 0.044	0.0325 ± 0.022	0.0424 ± 0.162	0.036 ± 0.113	6.67%	2	2
Rajshahi	3	14	1.115 ± 0.054	0.059 ± 0.028	0.1106 ± 0.241	0.0741 ± 0.168	13.33%	5	5
Mymensingh	3	11	1.032 ± 0.031	0.016 ± 0.016	0.0212 ± 0.122	0.0148 ± 0.081	3.33%	1	3

3.2. Population differentiation and genetic distance

AMOVA data revealed a significant ($P=0.001$) genetic difference among the five populations. The genetic differentiation was higher among populations (79%) than within population (among individuals) in each particular population (21%). This result shows that populations of different regions are genetically distinct but samples within a population taken from a particular region are genetically more close to each other.

Nei's measures of genetic identity and genetic distance among populations are shown in Table 4. The five populations of *Lantana camara* L. showed genetic distance among them ranging from 0.215 to 0.664. The genetic distance between population of Rajshahi and Mymensingh was the biggest (0.664) and that between populations of Dhaka and Sylhet was the smallest (0.215).

Table 4 Nei's measures of genetic identity and genetic distance. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among populations of *Lantana camara* L. from Dhaka, Sylhet, Chattogram, Rajshahi and Mymensingh divisions

Population ID	Dhaka	Sylhet	Chattogram	Rajshahi	Mymensingh
Dhaka	-	0.8063	0.7339	0.7416	0.6147
Sylhet	0.2154	-	0.6452	0.6063	0.5497
Chattogram	0.3094	0.4382	-	0.5980	0.6380
Rajshahi	0.2990	0.5004	0.5142	-	0.5147
Mymensingh	0.4866	0.5985	0.4495	0.6643	-

3.3. Cluster analysis and Isolation by distance

Data obtained from PCoA revealed a genetic pattern in which the individuals were assigned into three separate groups, one clearly representing the Mymensingh population (M1-M3) and the other Rajshahi (R1-R3) population indicating that these two are relatively distinct from all other sampled populations. The bi-plots of Dhaka (D1-D5), Sylhet (S1-S3) and Chattogram (C1-C3) populations were in the same group i.e. relatively close to the main cluster of samples. In PCoA, the overall cumulative variation explained by the PC1, PC2 and PC3 was 30.56%, 51.16% and 70.41%, respectively (Figure 2).

A significant positive correlation ($P = 0.001$) was found between genetic distance and geographic distance of all the samples collected, indicating an isolation by distance pattern of divergence (Figure 3). Mantel test yielded a correlation coefficient for the two data matrices by random permutation, with a range from -1 to $+1$. As this result was significant at the 5% level the observed correlation co-efficient ($R^2 = 0.659$) was found more extreme (closer to $+1$ or -1).

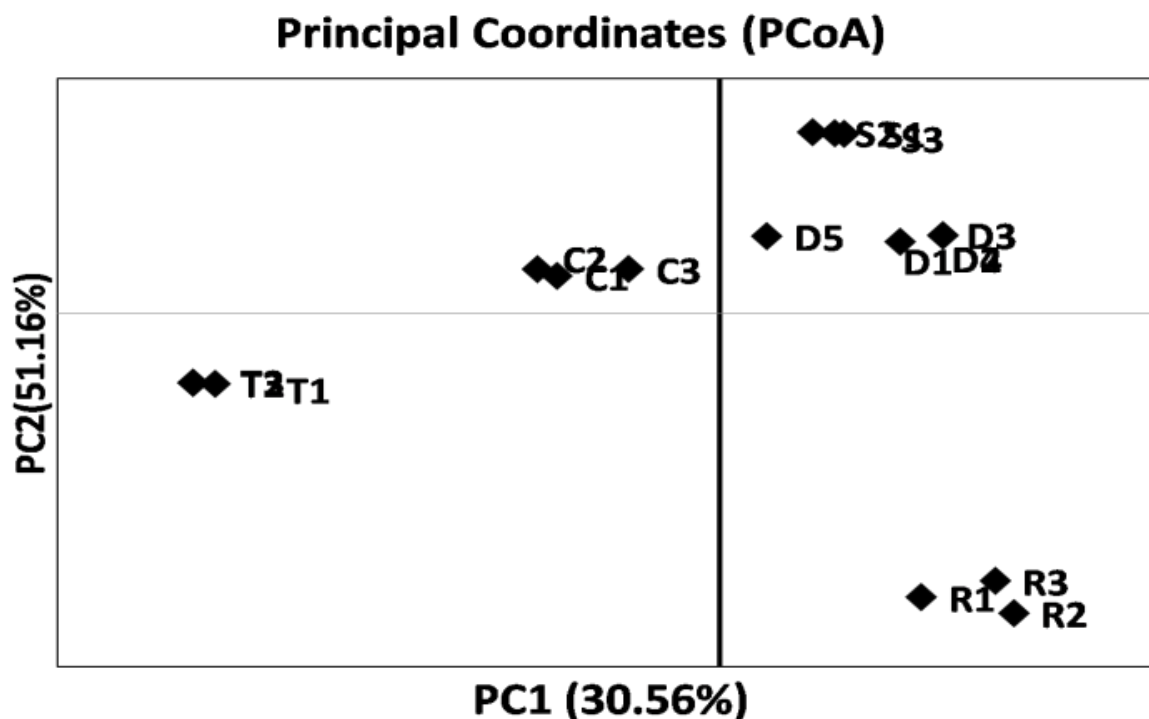


Figure 2. Biplots obtained from Principal Coordinate Analysis (PCoA) for microsatellite data of the five populations of *Lantana camara* L. from Dhaka (D), Chattogram (C), Rajshahi (R), Mymensingh (M) and Sylhet (S) administrative divisions in Bangladesh

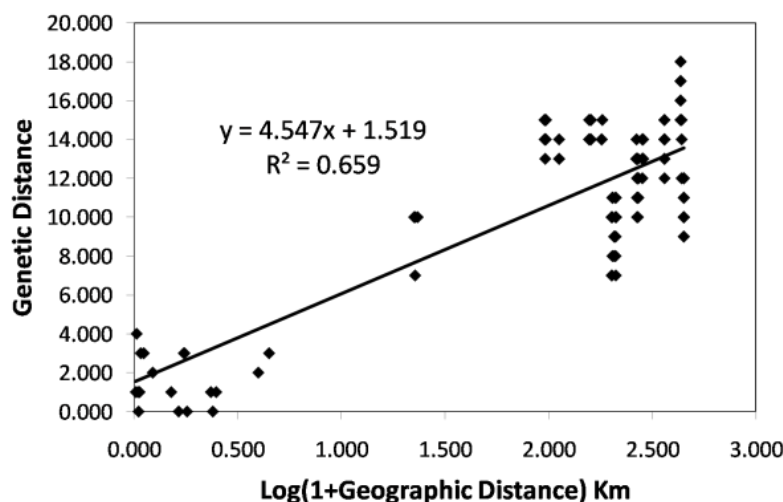


Figure 3. Mantel test showing the relationship between pair-wise genetic distance and log transformed geographic distance for microsatellite loci ($y = 4.547x + 1.519$, $R^2 = 0.659$, $P = 0.001$).

4. DISCUSSION

The microsatellite profiles analyzed in the present study revealed a high allelic richness within and between populations of lantana found in the different geographical regions of Bangladesh. Presence of high genetic diversity in this species was also revealed by the Shannon's information index and the percentage of polymorphic loci (Table 3). High genetic diversity in the invasive plant species was also reported by other studies (Roman and Darling 2007). Such high genetic diversity in invasive plants was attributed to the effects of multiple founders in the invaded range of the invaders (Mengistu and Messersmith 2002; Kolbe et al. 2004; Marrs et al. 2008; Henry et al. 2009). Multiple introductions was a source of high level of genetic diversity in the introduced range of invasive populations (Maron et al. 2004) than that in the native range (Kolbe et al. 2004). Liang et al. (2008) estimated comparatively high genetic diversity of invasive *Coreopsis grandiflora* in China that was found consistent with other studies in some invasive species (Meekins et al. 2001; Mengistu and Messer smith 2002). In addition to high allelic richness and other diversity indices, the number of private allele found in different populations of lantana indicated the probability of multiple introductions of this species. Number of unique alleles ranging from 3 to 5 was found with a total of 16 in 17 plant samples suggesting the existence of multiple sources for invasive populations of lantana in Bangladesh. No single source population could account for this high number of private alleles as such alleles are likely to owe their origin to divergent founders from different geographic sources. Introduced populations generally experienced a loss of some rare alleles because of the genetic bottleneck occurring at introduction (Neiet al.1975). Therefore, during bottleneck, rare alleles face a particularly high chance of loss but multiple introductions from distinct genetic sources carrying diverse sets of alleles could result in higher genetic diversity in the invasive populations despite the loss of alleles in the separate founding populations (England et al. 2003). As multiple introductions were expected to carry a diverse array of alleles including private alleles from divergent source populations, such introductions could give rise to high population differentiation, higher number of alleles, increased allelic richness and private polymorphism in the introduced range (Keller and Taylor 2010). Thus, the high genetic diversity of lantana found in the study area generally painted the picture of multiple introductions of this species in the territory of Bangladesh.

Analysis of population structure also indicated multiple introductions of lantana by showing the significant genetic differentiation with higher (79%) among population and lower (21%) within population variation. The high among population differentiation of introduced lantana populations suggested that they were founded by genetically distinct sources. The result was consistent with the other study (Durka et al. 2005) that reported only 25% variation residing within population and 71%variationamong populations within continent. They also reported that even when two continents were analyzed separately 79% of genetic variation was partitioned as among population variation in the introduced ranges and the amount of differentiation among populations suggested introductions of garlic mustard (*Alliariapetiolata*) from several native populations to North America.

PCoA is a powerful multivariate analysis which is often used to examine whether populations show clustering on the basis of genetic data. Results of the present study revealed that there were multiple divergent genetic clusters among the populations of lantana where the populations of Dhaka, Sylhetand Chattogram were more close to each other although that of Mymensingh and Rajshahi were somewhat genetically distinct from each other. This result indicated that genetically distinct groups of lantana were introduced to various locations in Bangladesh. After introduction, subsequent spread led to the mixing of individuals from different

groups (Facon and David 2006; Kolbe et al. 2008). This admixture of different groups resulting from multiple introductions could infuse the invasion with novel alleles and the generation of inter-population hybrids.

A significant positive relationship between geographic and genetic distance revealed by Mantel test (Figure 3) also demonstrated the evidence for multiple introductions of lantana in the territory of Bangladesh. Further, such a significant positive correlation suggested a pattern of gene flow conforming to the Isolation By Distance model since a non-significant relationship between geographic and genetic distance would indicate a lack of inter-population gene flow and/or genetic drift within populations and a negative isolation by distance pattern indicated reduced genetic diversity due to founding events during range expansion (Wallace 2002). In India, Ray and Quader (2014) reported multiple introductions of lantana with subsequent range expansion and gene flow. The western, northern and eastern parts of Bangladesh share border with five Indian states stretching up to 4,156 km and there is a great possibility that lantana was introduced in Bangladesh from the neighboring country.

A moderately strong pattern of isolation by distance observed in the present study confirmed very recent multiple introductions of lantana populations in different geographic regions of Bangladesh. A significant positive correlation along with allelic richness, diversity index, relationships among divergent genetic cluster, among population and within population differentiation indicated limited gene flow (Sexton et al. 2013) and limited gene flow promoted genetic divergence over long distances (Peterson and Denno 1998). However, lower genetic distance between Dhaka, Sylhet and Chattogram and clustering together indicated that there was, perhaps, gene flow among them after introduction which resulted in slight erosion of genetic diversity as the invasive range expanded outward from the initial point of introduction.

5. CONCLUSION

The present study suggested the possible role of multiple introductions in elevating genetic diversity and further substantiated the hypothesis that multiple introductions could contribute in gaining genetic diversity and such increased genetic diversity might result into rapid spread of invasive species as evident from lantana invasion in Bangladesh. Data obtained in the present study also emphasized the use of exploring genetic architecture of invasive species and its relationship with evolution that would help to establish proper management protocol to control unwanted invasion of invasive species like *L. camara* L. in introduced areas.

Author's contributions

All authors contributed equally.

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